Shoichiro Tsukita: a life exploring the molecular architecture of the tight junction

On December 11, 2005, Shoichiro Tsukita died at the young age of 52, after 14 months of treatment for cancer. Early in his career, Tsukita succeeded in isolating and purifying the adherens junction with his wife Sachiko, an accomplishment that he followed up with an impressive series of discoveries of cell adhesion and cytoskeletal molecules, including what may have been his greatest contribution to the field, the identification of occludin and the claudin family of molecules, which were watershed discoveries in the study of the molecular nature of tight junctions.

As a graduate student in the University of Tokyo Medical School Department of Anatomy, Tsukita found an able mentor in Harunori Ishikawa, who himself had earned acclaim for demonstrating the general prevalence of actin in nonmuscle cells while working in Howard Holtzer's lab in the late 1960s. Although he had always had a keen interest in molecular biology, as an undergraduate Tsukita was drawn to histology, and his first interest was to identify axonal transport mechanisms. He revealed for the first time, by electron microscopy, that different types of membranous structures were involved in anterograde and retrograde axonal transport, a pioneering advance in this field. He also made substantial contributions to the fields of muscle contraction and ciliary movement by combining the use of electron microscopy and rapid freezing in liquid helium. This was a critical formative period for Tsukita, as it was here that he first became excited about the possibilities for studying problems of morphology via a molecular biological approach, and even more importantly, it was during this period that Sachiko became his inseparable partner both in the lab and in life.

My first introduction to Tsukita's work in the field of cell adhesion came in 1986 at a Japan Society for Cell Biology meeting, where Sachiko, in collaboration with Shoichiro, was presenting their discovery of the desmosomal protein, desmocalmin. In those days, the desmosome was still terra incognita at the molecular level, and I was struck by the energy and enthusiasm of the Tsukitas, who were about ten years younger than I, in exploring this uncharted terrain. It would not be long, however, before they turned from the desmosome to the adherens junction, a cell–cell adhesion structure that Shoichiro and Sachiko were the first to isolate intact, from preparations of rat liver hepatocytes (Tsukita and Tsukita, 1989). These purified adherens junctions (Fig. 1) represented something of a mother lode for Tsukita, whose scrutiny of their molecular composition yielded a cornucopia of important discoveries (Fig. 2), and indeed

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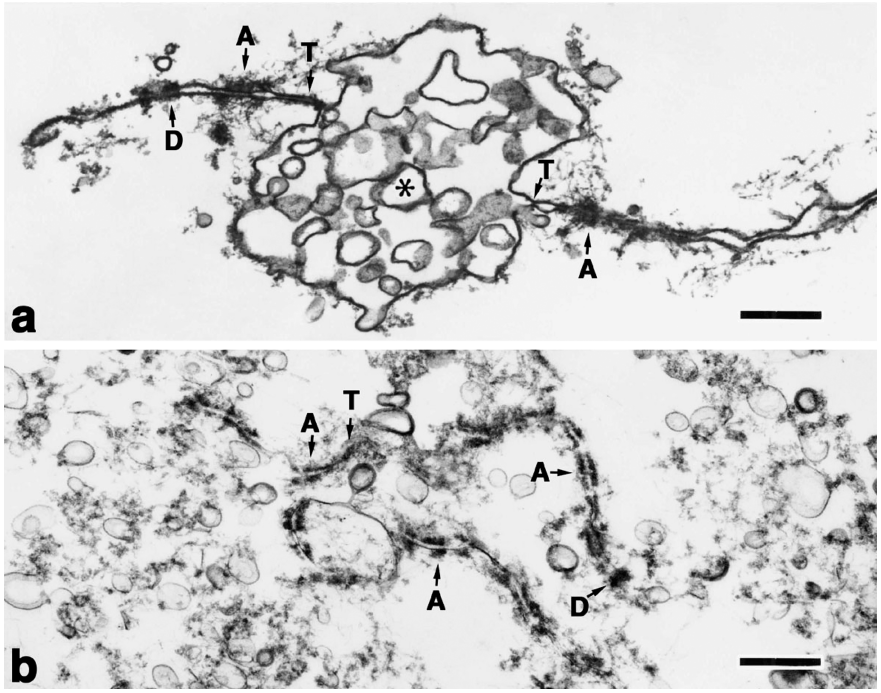

Figure 1. **Ultrathin sectional electron microscopic images of bile canaliculi and junction fractions.** (a) The isolated liver bile canaliculus (*) associated with tight junctions (T), adherens junctions (A), and desmosomes (D). (b) The junction fraction with tight and adherens junctions, and desmosomes, recovered after treatment of the bile canaliculi fraction with NP-40. Bar, 0.5 μm. From *J. Cell Biol.* 108:31–41. 1989.

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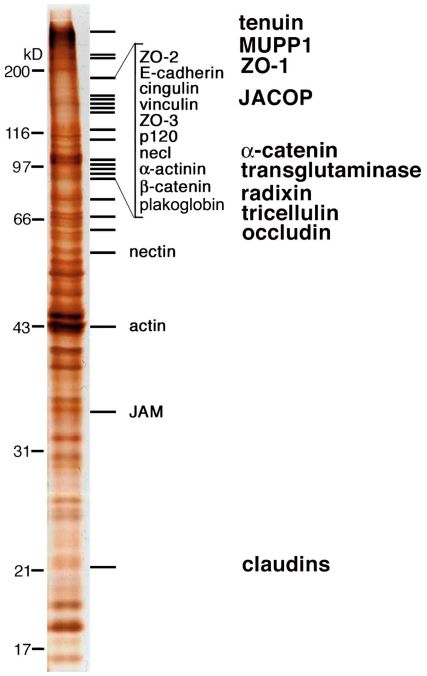

Figure 2. **SDS-polyacrylamide gel electrophoretic banding patterns of the junction fraction prepared from liver.** The main components of tight and adherens junctions are shown, as identified in the Tsukita (right column) and other laboratories. Courtesy of Yugi Yamazaki and Sachiko Tsukita.

launched him down a path that he would pioneer for the rest of us throughout the remainder of his career.

The first molecule that the Tsukitas teased out of the complexity of the adherens junction (AJ) fraction was radixin, one of the founding members of the eponymous ERM family; Sachiko has gone on to advance the study of this molecule in her own work. α-Catenin was the next molecule to emerge from the AJ trove, a finding that contributed immensely to our understanding of the cadherin cell adhesion machinery. The subsequent discovery of ZO-1, a known tight junction (TJ) protein, in what until then had been thought to be a pure AJ fraction led Tsukita to surmise that the fractions were in fact enriched with TJ components as well. Tsukita set Mikio Furuse, a gifted graduate student in his lab, to the task, and in 1993 the team developed a monoclonal antibody from their AJ preparation that recognized a protein in the tight junction. They cloned this 4-pass transmembrane protein and christened it occludin (Furuse et al., 1993), a name taken from the TJ's alternative appellation, zonula occludens, marking the identification of one of the first TJ-specific membrane proteins. Tight junctions are adhesive structures found in the most apical regions of epithelial cell layers, where they prevent the unregulated passage of matter between cells (Fig. 3). A number of tight junction cytoplasmic factors had been described relatively early on, but for many years its membrane organization remained unresolved at the molecular level.

After the excitement of the occludin discovery, Tsukita was later to experience disappointment on finding that tight junctions form even in cells in which the occludin gene had been deleted. I still remember his struggle to account for the inexplicable phenotype of the occludin knockouts as he prepared the results for publication. At the same time, he and Furuse had already decided that there must be another membrane protein at work in the tight junction, a prediction Furuse proved correct by sifting through a subfraction of occludin-positive membrane from the purified adherens junction. His search yielded a pair of novel proteins, which he named claudin-1 and -2 (Furuse et al., 1998a). As predicted, the claudins were shown to localize at the tight junction, and even more happily, caused fibroblasts (which do not normally form tight junctions) to form characteristic TJ strands when ectopically expressed (Fig. 4) (Furuse et al., 1998b), a demonstration that provided the conclusive evidence in the long search for an essential TJ membrane protein.

Research into claudins blossomed rapidly into a fertile and active field of molecular biology, and this protein family is now known to include at least 24 members, highlighting a hitherto unsuspected diversity among tight junctions that continues to challenge and reward investigators. The discovery of the claudins has led to a number of new revelations. As just one example, when investigators found that claudin-1 knockout mice exhibited an unusual dry skin phenotype (which was remarkable in that the skin was traditionally believed to lack tight junctions), it led to the discovery of a new role for this form of cell adhesion in preventing water loss through the body surface by a TJ-dependent barrier (Furuse et al., 2002). In the blood–brain barrier as well, a claudin (this time, claudin-5) was again shown to be indispensable to physiological function (Nitta et al., 2003). Work by other scientists has shown that genetic defects in other claudin proteins can lead to inherited disorders including kidney diseases (Simon et al., 1999; Müller et al., 2003) and deafness (Wilcox et al., 2001), demonstrating the impact of Tsukita's work on the biomedical community.

Shoichiro Tsukita and I frequently attended meetings together. One in particular stands out in my memory. We were in Lake Placid, New York for the 14th International Symposium on Cellular Endocrinology: Cell Signaling and the Cytoskeleton, and the night before Tsukita's talk it sleeted so hard that the meeting venue suffered a total blackout. The next morning, with the power still out, we made our way down the hall in complete blackness for his session, which it had been decided would be given using only a blackboard for visuals. I think it's safe to say that most structural biologists would rather give a talk without their clothes on than without their slides, but Tsukita got up and made his presentation armed only with a piece of chalk. I will forever remain impressed by the example of his "chalk talk", and perhaps a bit chastened by his demonstration that even a great discovery such as he described that day can be conveyed so fully using such humble tools. At Keystone Symposia, we often hit the

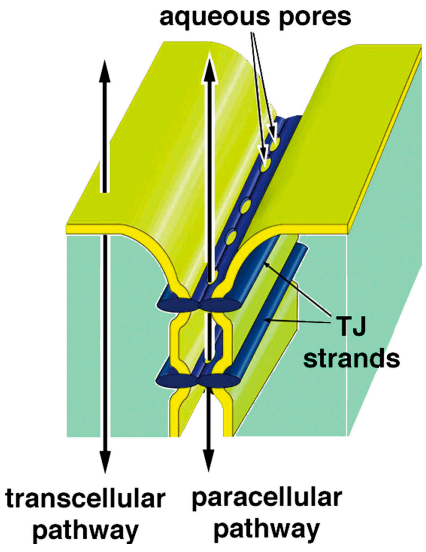

Figure 3. **Schematic drawing of tight junction.** From *J. Cell Biol.* 149: 13–16, 2000.

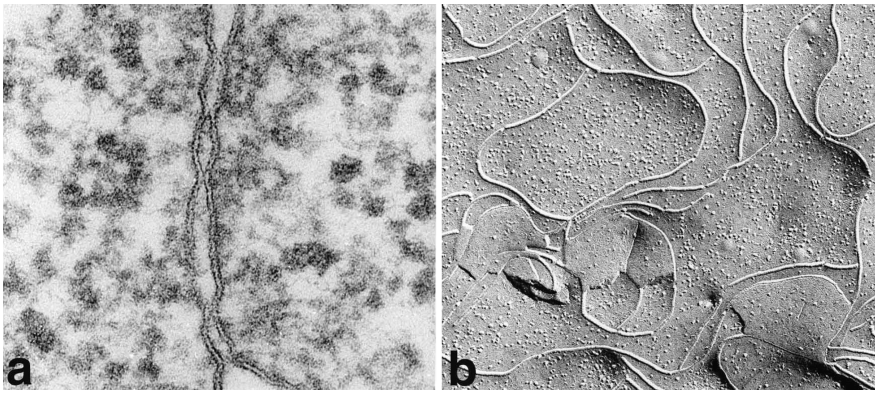

Figure 4. **Ultrathin-section and freeze-fracture electron microscopy of L cells expressing exogenous claudin.** (a) A series of apparent contacts between the plasma membranes of adjacent cells, where the intercellular space was completely obliterated. From *Curr. Biol.* 9:1035–1038, 1999. (b) A network of tight junction strand-like structures, reconstituted at cell–cell contact planes. Courtesy of Hiroki Sasaki.

slopes together, and he somehow managed to take advantage of the breaks between sessions to ski down every slope.

Even during his illness, Tsukita was engaged in discovering an important new molecule, tricellulin (Ikenouchi et al., 2005). Claudins function at tight junction regions that involve only two cells, but it is known that cell layers also include junctions where three cells meet: what are known as tricellular contacts. Just as with their two-cell cousins, these tricellular TJs must form a functional epithelial barrier. During a comprehensive screen of 4-pass transmembrane proteins, Junichi Ikenouchi, a grad student in Tsukita's lab, uncovered a molecule that localized at tricellular contacts, adding yet another achievement to the list of discoveries by Tsukita's lab of molecules whose existence was predicted by no more than the fact that "they ought to be there."

The acuity of Tsukita's scientific instincts was formidable, and he had an equal gift for observation. Findings from histological studies may seem unexciting at first glance to some, and I suspect that they often meet with a colder welcome from peer reviewers than do molecular biology manuscripts when submitted for publication. But at the end of the day, structures tend to have the final say in determining an experiment's validity, and I'm certain that Tsukita's work will live on in perpetuity in textbooks of cell biology. The great majority of his many important primary research articles were published in the *Journal of Cell Biology*.

Shoichiro Tsukita was first diagnosed with pancreatic cancer after a routine health exam in October 2004 and, supported by his many friends and loved ones, he endured this affliction resolutely. I visited him on the last day of November (less than two weeks before his death), and he was in the lab as always to greet me. Perhaps due to the medication it wasn't easy for him to talk, but Sachiko took the time to describe some of their unpublished work, including a paper he was working on even then. I could only marvel at his determination to see his research through to publication, and although his lab will no doubt continue on its enormously productive course, it is deeply saddening to think that he will no longer be there to contribute. On that visit, I knew that his condition was deteriorating and planned to stay only for thirty minutes or so, but as we talked we lost track of time and I ended up staying for more than one and half hours. He continued to go to his lab even after that, and I have heard that even six days before his death he was up late discussing research with coworkers. Nothing, not even cancer, could keep him from the work he loved; indeed, Sachiko told me it was his wish to die while enjoying science by working, thinking, and typing out a manuscript for publication. This was a scientist's scientist, maintaining a true sense of curiosity and wonder to the last. That he was able to turn his life's work in research into an elegant and inimitable work of art was, I believe, a true source of happiness for him, and I think I can speak for the entire community in saying that we look forward to the members of his lab carrying on in his proud tradition and committing themselves to the prodigious example he set in his life of scientific discovery.

I would like to thank Dr. Ira Mellman of the Journal of Cell Biology for providing me with the opportunity to contribute this obituary, and Sachiko Tsukita and Mikio Furuse for kindly providing figures from Dr. Tsukita's research. I also thank Douglas Sipp for his help in preparing this manuscript.

Masatoshi Takeichi
takeichi@cdb.riken.jp

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